Current Biology Vol 16 No 21
R918

Dispatches

Developmental Biology: Holding Pattern for Histones

New research on lipid droplets in *Drosophila* embryos has led to the surprising conclusion that these poorly understood organelles have a novel role as a regulated storage depot of maternally supplied proteins, particularly histones.

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Lipid droplets are largely uncharacterized organelles that play an important role in the *Drosophila* life cycle by providing the developing embryo with a source of energy and building materials for membrane synthesis. Proteins associated with lipid droplets function to distribute lipid droplets throughout the embryo, and to regulate the release of stored lipids for further metabolism. As reported recently in *Current Biology*, Cermelli *et al.* [1] have carried out the first detailed analysis of the protein composition of lipid droplets isolated from *Drosophila* embryos. The unexpected finding that the lipid droplets contain abundant copies of histones H2A, H2Av and H2B has revealed a novel role for these unsung organelles as a transient storage depot of maternally supplied proteins.

From the time of deposition of the egg to the hatching of the first larval instar when feeding becomes possible, the *Drosophila* embryo must rely upon stored sources of energy. During oogenesis, triacylglycerols, an efficient form of stored energy, are packaged into tiny lipid droplets in nurse cells prior to transfer into the egg. These lipid droplets contain cores of neutral lipids, triacylglycerol and cholesterol ester, and are covered with a phospholipid monolayer. Efficient packaging of this energy supply requires the expression of maternally produced Lipid Storage Droplet protein 2 (LSD2), a protein that coats the tiny lipid droplets in oocytes and embryos [2].

LSD2 is an insect relative of perilipin, an abundant protein that coats the much larger lipid droplets of the fat-storing cells (adipocytes) of mammals. Studies in cultured mammalian cell models and mice genetically altered to lack perilipin have shown that perilipin plays important roles in stabilizing fat stores and regulating hydrolysis of triacylglycerols to release fatty acids that are metabolized by many tissues of the body to produce energy. When food is plentiful, perilipin is minimally phosphorylated and acts as a barrier to lipolysis [3–6], thus promoting the storage of excess calories as triacylglycerols in adipose tissue. When energy is required as a result of fasting or extended exercise, perilipin becomes multiply phosphorylated and facilitates the access of cytosolic lipases to stored triacylglycerols for lipolysis [4–10].

Similarly, studies in genetically altered *Drosophila* have shown that LSD2 helps to maximize storage of triacylglycerol in larval and adult fat bodies [2,11], and thus, extends the lifespan of adults when food is scarce [11]. Cermelli *et al.* [1] report that LSD2 is an abundant protein component of lipid droplets isolated from early *Drosophila* embryos and, like perilipin, is multiply phosphorylated in a regulated manner [12]. The phosphorylation of LSD2 correlates with increased lipid droplet movement throughout the embryo [12], but it may also be an important factor in the control of lipolysis to release fatty acids for β-oxidation to produce energy for the developing embryo. Fatty acids and partially hydrolyzed lipids also serve as important substrates for the synthesis of phospholipids required for building cellular membranes.

Early *Drosophila* embryogenesis is unusual among multicellular organisms in that rapid nuclear division during the first three hours after deposition of the egg is not accompanied by cell division. Twelve nuclear divisions generate more than 4000 nuclei which share a common cytoplasm in a syncytial blastoderm. In the later cycles, the nuclei migrate to the periphery of the embryo and cellularization occurs there at cycle 13. The histone proteins required to assemble thousands of copies of the *Drosophila* genome into chromatin during the early nuclear divisions are derived from maternal histones deposited in oocytes, and from translation of maternal mRNAs [13]. Because free histone proteins in excess of available DNA binding sites are potentially toxic [14], the problem becomes: how to rapidly obtain the histones needed during the first 12 nuclear divisions, and where to store these extra histones so that they do not cause trouble?

Surprisingly, lipid droplets appear to provide a safe haven for embryonic histones. Cermelli *et al.* [1] found that newly deposited *Drosophila* embryos are loaded with enough maternally derived histones to package thousands of copies of the genome into chromatin. A large percentage of the total mass of histones 2A, 2Av, and 2B (H2A, H2Av, and H2B) are bound to lipid droplets (Figure 1). Furthermore, the content of histones on lipid droplets decreases during the first six hours of embryogenesis, consistent with release of the histones for use during rapid nuclear division in the developing embryo. These provocative findings raise a number of interesting questions.

Where in the embryo are the other core histone components of chromatin stored? Correct assembly of chromatin following DNA replication requires equal

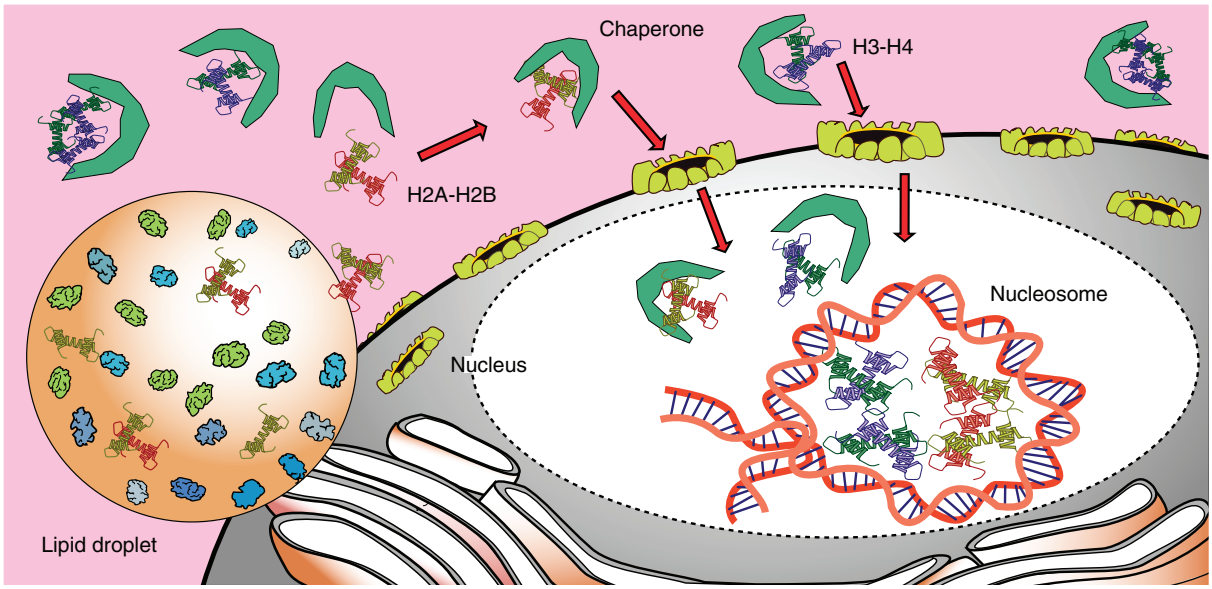

Figure 1. Lipid droplets in *Drosophila* embryos serve as a storage depot for histones H2A and H2B.

H2A and H2B are released from lipid droplets and transported into the nucleus, most likely escorted by a chaperone protein. Within the nucleus, H3–H4 tetramers assemble onto genomic DNA, followed by the association of two H2A–H2B dimers. The resulting nucleosome is the basic unit of chromatin packaging. (Illustration by R. Hasney.)

amounts of four core histones: H2A, H2B, H3, and H4 (Figure 1). H3 and H4 readily form histone tetramer–DNA complexes [15,16]. H2A and H2B form stable dimers, but only assemble into the complex after the H3-H4 tetramer is in place on genomic DNA. Two H2A-H2B dimers are needed to complete the nucleosome, the basic unit of DNA packaging into chromatin. Rapid nucleosome assembly requires that the embryo has an equivalent supply of all four core histones. If the lipid droplet is the source of only H2A and H2B, then where are H3 and H4? H3 and H4 must also be sequestered somewhere in the embryo, perhaps bound to chaperone proteins, and coordinately made available with the H2A and H2B.

Are H2A and H2B associated with lipid droplets in dimeric or monomeric forms? Stable H2A–H2B dimers form readily in solution, but rough quantification of the histone content of isolated lipid droplets indicates that H2A is present at twice the mass of H2B [1], suggesting association of H2A monomers with lipid droplets. Similarly, is the variant histone H2Av monomeric, or complexed with H2B on lipid droplets?

Why lipid droplets? The protein or lipid composition of the lipid droplets may hold clues to the affinity of histones for these organelles. Incubation of lipid droplets with detergents releases LSD2, but not H2B, into solution [1]. By contrast, solutions that reduce electrostatic interactions strip H2B off of the lipid droplets, while leaving LSD2 bound. Thus, the histones are bound by electrostatic interactions with the droplet, not through hydrophobic interactions or electrostatic interactions with LSD2, the most abundant structural protein of the lipid droplets.

It is possible that the highly basic histones interact with the negatively charged head groups of the phospholipids coating the droplets. However, the phospholipid composition of the lipid droplet is likely similar to that of other cellular membranes [17], particularly endoplasmic reticulum, which is thought to be the site of lipid droplet formation. Additionally, the selective binding of H2A and H2B to lipid droplets is difficult to explain by a general charge interaction of histones with phospholipids. Further experimentation is required to determine whether the specificity of H2A and H2B association with lipid droplets is due to a unique phospholipid or protein composition, or perhaps promoted by the uniquely curved droplet surface.

What regulates the release of H2A and H2B from the embryonic lipid droplets, and how do they get to the nucleus? The availability of maternally supplied histones must be carefully controlled to facilitate correct packaging of chromatin during rapid DNA replication that precedes nuclear divison, while preventing poisoning of the embryo. Studies of chromatin assembly have elucidated roles for chaperone proteins such as NAP1 in escorting H2A and H2B into the nucleus and depositing histones onto DNA [16]. Are these chaperones also associated with lipid droplets, are they rapidly synthesized in concert with embryonic DNA replication, or are they waiting in the cytoplasm to assist in pulling the histones off of the lipid droplets at exactly the correct time? And finally, is the regulated release of histones coupled to the hydrolysis of triacylglycerols to provide fatty acids for β-oxidation and ATP production? One thing is certain: these novel findings are likely to be the source of much

debate and a flurry of new investigation into the biology and biochemistry of lipid droplets.

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DOI: 10.1016/j.cub.2006.09.052

Homing Behavior: Decisions, Dominance and Democracy

When flying in pairs, pigeons with different preferred paths back to the loft often agree on a joint route, and in doing so get home faster than either would have done on its own.

James L. Gould

We sometimes hear that two halfwits do not make a whole. Among pigeons at least, this old saw may be incorrect. Pigeon homing is almost always studied by releasing birds singly some tens or hundreds of kilometers from the home loft, and then measuring a vanishing bearing. A persistent but little-known problem with this protocol is that pigeons prefer to home in the company of other pigeons, and some will alight in a tree near the release site and wait for a colleague to begin the journey home. For the experimenter, this can invalidate two potential data points, and most workers come armed with ways of encouraging the waiting bird to depart.

The apparent desire not to home alone reflects a strong social tendency in pigeons. Sociality is rare among animals. One of the selective forces that makes group living advantageous for pigeons is predator detection and escape: the distance at which birds of prey are spotted increases dramatically with foraging group size, and the chance of a predator successfully taking even a single bird from a feeding flock plummets at the same time. This same preference is seen in flight: birds released in a pigeon race form up into a group and set off to their various lofts as a mob with (at the outset) a single vanishing bearing.

But the coherent initial flight of a flock of birds must somehow be created by individuals whose vanishing bearings when released as individuals are spread out over 60° or even 90°. Is there a leader, or some sort of averaging, or just a simple nearest-neighbor rule that acts as a kind of behavioral gravity or glue? Or perhaps some combination of these alternatives is at work. This is a question the answer to which may be relevant to a variety of behaviors requiring group coordination.

Previous work by Michener and Walcott [1], involving low-resolution air and radio tracking of birds, showed that pigeons released at relatively long distances from the loft often flew quite indirect routes home, and would usually find a very different